

# Comparison of Sublingual and Oral Prazepam in Normal Subjects

## II. Pharmacokinetic and Pharmacodynamic Data

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**Abstract.** The pharmacokinetic profiles of oral and sublingual administrations of prazepam 20 mg to 5 normal volunteers were compared in order to explain the clinical observation that sublingual prazepam appears to exhibit sedative properties when compared to the same dose of oral prazepam. Blood samples for pharmacokinetic evaluation were collected just before drug intake and 7.5, 15, 22.5, 30, 45, 60, 90 min, 2, 3, 5, 6, 7, 8, 9, 10 and 24 h after drug intake. The study was performed in double-blind and crossover conditions. Serum levels of prazepam and its major metabolite N-desmethyl-diazepam were measured by HPLC. No prazepam was detected at a concentration higher than 20 ng/ml (limit of detection) whereas N-desmethyl-diazepam reached concentrations around 140 ng/ml. To correlate this observation with the clinical data, the affinity of prazepam and N-desmethyl-diazepam was compared measuring their ability to displace 50% of <sup>3</sup>H-flunitrazepam bound to benzodiazepine receptors contained in synaptosomal preparation obtained from rat brain. N-desmethyl-diazepam was 17-fold more potent than prazepam. This data suggests that prazepam is a pro-drug which is transformed to the active compound N-desmethyl-diazepam and that the difference in clinical observation with both administrations could be correlated to N-desmethyl-diazepam concentration-time curves. Nevertheless, the comparison of the area under the N-desmethyl-diazepam serum concentration-time curves, the maximum concentrations, the times when the maximum concentrations were observed and the times needed to detect a significant level after oral and sublingual administration did not show statistical difference. This lack of statistical difference between the two administrations hampers a direct pharmacokinetic interpretation of the clinical observation that sublingual administration of prazepam appears to exhibit more subjective sedative properties than oral administration.

### Introduction

Prazepam is a member of the 1,4-benzodiazepine group of drugs. It has an N-1-cyclopropylmethyl group in place of the N-1-methyl group of diazepam. Prazepam and diazepam form a common metabolite (N-desmethyl-diazepam) by N-dealkylation at the N-1 position. Although earlier workers [DiCarlo et al., 1970; Vesell et al., 1972] suggested that the presence of the cyclopropylmethyl group on the molecule at position 1 hindered the

metabolism of the molecule at this point, it is accepted now [Smith et al., 1979; Ochs et al., 1984; Mura et al., 1987] that prazepam is rapidly transformed to its major metabolite N-desmethyl-diazepam. Indeed, the prazepam and the N-desmethyl-diazepam maximum concentrations after oral administration of 30 mg of prazepam are  $6.6 \pm 7.9$  and  $321 \pm 76$  ng/ml respectively [Smith et al., 1979].

On the other hand, recent clinical results reported by Ansseau et al. [1987] have shown that a single dose of

prazepam 20 mg yields more subjective sedation when administered sublingually than orally. The authors have suggested that the differences in subjective sedation level between sublingual and oral prazepam could correspond to differences in absorption rate. The present study shows the pharmacodynamic and the pharmacokinetic aspects of this comparison.

The first step of the study was to compare the potential activity of both prazepam and N-desmethyl-diazepam measuring their affinity for the benzodiazepine receptor. This comparison was based on the relative ability of benzodiazepines to displace 50% of  $^3\text{H}$ -flunitrazepam bound to the benzodiazepine receptors in an *in vitro* test.

The second step was to compare the serum level-time curves of the active compounds during the earliest period following oral and sublingual intakes.

## Subjects, Materials and Methods

### Pharmacodynamic Study

#### Materials

$^3\text{H}$ -flunitrazepam (86 Ci/mmol) was purchased from the Radiochemical Center (Amersham, UK). Diazepam, N-desmethyl-diazepam, oxazepam and flunitrazepam were obtained from Hoffmann-LaRoche (Basel, Switzerland). Prazepam was obtained from Parke-Davis (Brussels, Belgium).

#### Methods

**Synaptosomal Membrane Preparation.** This preparation was obtained as described by Möhler and Okada [1977]. Female Wistar rats (200–300 g) were decapitated and their brains were rapidly removed and placed in ice-cold 0.32 M sucrose. One minute later, the whole brain without cerebellum was homogenized for 40 s in 15 volumes of ice-cold 0.32 M sucrose in a Potter-Elvehjem homogenizer fitted with a Teflon pestle and centrifuged at 1,000 g for 10 min. The supernatant was centrifuged at 48,000 g for 20 min. The resulting pellet was suspended in Krebs-Tris buffer pH 7.4. The concentration of protein determined according to Lowry et al. [1951] was adjusted to 15 mg/ml. This crude preparation (P2 fraction) was stored at  $-18^\circ\text{C}$ .

**Bindings Assays.** The binding assays were carried out as described by Jacqmin et al. [1986]. The assays were performed at room temperature ( $22^\circ\text{C}$ ). The synaptosomal preparation (1.5 mg protein) was incubated with  $^3\text{H}$ -flunitrazepam (0.3 pmol) in Krebs-Tris buffer pH 7.4 in the presence of various concentrations of the drug to be studied, in a final volume of 1.8 ml. The endogenous GABA concentration in the incubation medium has been valued to  $6.46\ \mu\text{M}$  by a radioreceptor assay [Mousah et al., 1987]. After 30 min incubation, separation between bound and free drug was performed by centrifugation at 1,000 g for 10 min in plastic scintillation mini-vials. The supernatant was then discarded and the resulting pellet was dissolved in 3.5 ml of Aqualuma Plus (Lumac, Holland) by vigorous vortexing. The radioactivity was counted in a Packard counter model Tri-carb 2425 (efficiency 0.46).

The radioactivity bound in the presence of  $10\ \mu\text{M}$  flunitrazepam (nonspecific binding) was subtracted from all binding values. It amounted to 2,750 dpm, i.e. 10–15% of the total binding in the absence of displacing drugs.

The assays were performed in duplicate. The  $\text{IC}_{50}$  were calculated by logit-log graphical analysis from the means of duplicates (7 points). The results are expressed as means  $\pm$  standard deviation of (n) experiments.

### Pharmacokinetic Study

Subjects and general procedure are the same as described by Anseau et al. [1988].

#### Subjects

The study was performed in 5 healthy volunteers, members of the medical staff of the University Hospital of Liège, Belgium. All subjects had a normal clinical examination and a biological balance sheet within normal values. The personal details of the subjects are given in table I.

The volunteers had not taken any drug during the previous 2 months. Moreover, they were not allowed to use any medication throughout the study period. They were asked not to drink alcoholic beverages on the days of both sessions as well as the day before. The evening meal preceding both session days as well as the lunches of the session days were standardized and devoid of fat. The protocol was approved by the ethical committee of the University of Liège and all subjects gave their informed consent.

#### General Procedure

The methodology used was a double-blind crossover comparison of a single dose of sublingual or oral prazepam 20 mg in randomized order. In two different sessions, at a 2-week interval, the subjects first took orally a tablet of prazepam with 100 ml of water and second, placed another tablet of prazepam under their tongue, but whereas one was the active compound, the other was a placebo of the same appearance. Drug intake took place at 08.30 h, with the subjects fasting from the previous evening at 20.00 h until 12.30 h on the session day. Blood samples were collected in order to measure benzodiazepine levels just before drug intake and 7.5, 15, 22.5, 30, 45, 60, 90 min, 2, 3, 5, 6, 7, 8, 9, 10 and 24 h after drug intake. Assessment of psychological changes by visual analogue scales and computerized assessment of vigilance were also performed. The results of these assessments are reported in the clinical part [Anseau et al., 1987].

Table I. Personal details of volunteers

Volunteer	Sex	Age, years	Height, cm	Weight, kg
1	male	32	176	75
2	male	28	190	70
3	male	30	182	100
4	male	36	183	70
5	male	28	171	65

### Prazepam and N-Desmethyl-Diazepam Determination in Serum

The determination of prazepam and N-desmethyl-diazepam in serum was based on classical HPLC methods described in the literature [Mura et al., 1987; De Groot and Grotenhuis-Mullenders, 1984] and adapted to our apparatus.

**Chromatographic System.** Briefly, A Kratos Spectroflow 400 HPLC solvent delivery system was used, equipped with a Kratos Spectroflow 773 UV detector (wavelength of detection: 240 nm). A 25-cm-long, 4-mm ID column was used packed with 5  $\mu$ m Lichrospher 100 CH-8 (Hibar<sup>®</sup>, Merck, Darmstadt, FRG). The mobile phase was a mixture of acetonitrile (Alltech, Arlington Heights, USA) and water (55/45). The flow rate was 1 ml/min.

**Extraction Procedure.** A column extraction was performed using 3-ml reverse-phase octadecyl silane extraction columns (Bond-elut<sup>®</sup>, Analytichem International, Harbor City, USA) fitted to a Varian-10 extraction manifold (Varian Instrument group, Walnut Creek, USA). Preceding the actual extraction, the column was washed with 3 ml of methanol (Merck, Darmstadt, FRG), twice 3 ml methylene chloride (Merck, Darmstadt, FRG), twice 3 ml of methanol and twice 3 ml of 0.1 M sodium carbonate buffer (pH = 10.5). One mil-

liliter of the internal standard (diazepam 100 ng/ml free benzodiazepine serum) is brought to the top of the column followed by 1 ml of standard or sample. Following the elution, the water-soluble serum components are removed by washing the column consecutively with twice 3 ml of 0.1 M sodium carbonate buffer, 1 ml of a mixture of methanol and water (50/50) and 1 ml of a mixture of acetonitrile and water (40/60). The N-desmethyl-diazepam, prazepam and internal standard diazepam were eluted with 3  $\times$  1 ml of methylene chloride. The eluate was evaporated to dryness under nitrogen. The residue was dissolved in 50  $\mu$ l of methanol and an aliquot of 20  $\mu$ l was taken for the HPLC analysis.

Using this extraction procedure the percentage of recovery for diazepam, N-desmethyl-diazepam and prazepam was  $92 \pm 3.2$ ,  $96 \pm 2.8$  and  $89 \pm 3.1\%$  respectively. The limit of detection for N-desmethyl-diazepam and prazepam was 10 and 20 ng/ml respectively.

### Data Analysis

The area under the serum level-time curves up to 24 h was determined by trapezoidal rule integration. The areas under the serum level-time curves (AUC), the maximum concentrations ( $C_{max}$ ), the times when the maximum concentrations were observed ( $T_{max}$ ) and the times needed to detect a significant level of N-desmethyl-diazepam (lag-time) after oral and sublingual administration of prazepam 20 mg were compared by paired t tests, Wilcoxon test and by analysis of variance for two-way crossover study [Wagner, 1975]. The bioavailability of sublingual prazepam 20 mg versus oral prazepam 20 mg was analyzed using the test described by Westlake [1972, 1976; Spriet and Beiler, 1978; Hirtz, 1980].

## Results

The pharmacodynamic results presented in table II show that N-desmethyl-diazepam has a higher affinity than prazepam for the benzodiazepine receptor. The ratio between the  $IC_{50}$  was about 17. The time course of

**Table II.** Affinity of different 1,4-benzodiazepines to the benzodiazepine receptor expressed by their ability to displace 50% of <sup>3</sup>H-flunitrazepam bound to the receptor ( $IC_{50}$ )

Compound	$IC_{50}$ , nM	(n)
Flunitrazepam	$4.09 \pm 0.49$	(17)
Diazepam	$15.00 \pm 2.60$	(5)
N-desmethyl-diazepam	$24.90 \pm 0.60$	(3)
Oxazepam	$38.40 \pm 3.00$	(6)
Prazepam	$422.00 \pm 22.0$	(3)

The higher the  $IC_{50}$ , the lower the affinity of the benzodiazepine for the benzodiazepine receptor.

**Table III.** AUC,  $C_{max}$ ,  $T_{max}$  and lag-time in a double-blind crossover comparison of a single dose of sublingual or oral administration of prazepam 20 mg to 5 volunteers in randomized order

Subject	AUC, h $\times$ ng/ml				$C_{max}$ , ng/ml				$T_{max}$ , min				Lag-time, min			
	oral		sublingual		oral		sublingual		oral		sublingual		oral		sublingual	
3 (J.L.C.)	W1	1,272	W2	1,060	W1	70	W2	77	W1	120	W2	120	W1	45	W2	45
4 (M.A.)		2,439		1,761		136		130		300		120		30		45
1 (J.P.C.)	W2	2,542	W1	2,556	W2	144	W1	141	W2	90	W1	300	W2	22.5	W1	45
2 (D.E.)		2,407		2,381		127		138		120		120		22.5		22.5
5 (P.P.)		2,282		3,319		113		172		300		300		30		22.5

The comparison of AUC,  $C_{max}$ ,  $T_{max}$  and lag-time by paired t test, Wilcoxon test and analysis of variance for two-way crossover study did not show statistical difference between the two administrations.

W1 = Week 1; W2 = week 2.

N-desmethyl-diazepam serum concentrations after sublingual and oral administrations of prazepam to 5 volunteers is presented in figure 1. AUC,  $C_{max}$  and  $T_{max}$  are given in table III. The comparison of AUC,  $C_{max}$  and  $T_{max}$  by paired t test, Wilcoxon test and analysis of variance for two-way crossover study did not show any difference between the two administrations. The comparison of the areas under the serum level-time curves

up to 24 h showed that the bioavailability of the sublingual administration does not differ from the oral administration more than 7%. But taking into consideration the coefficient of variation of the residual error (17%) and the number of volunteers used in this study ( $n = 5$ ), the minimum difference that we could detect with 80% chance between both administrations, if it exists, would be 30% [Hirtz, 1980].

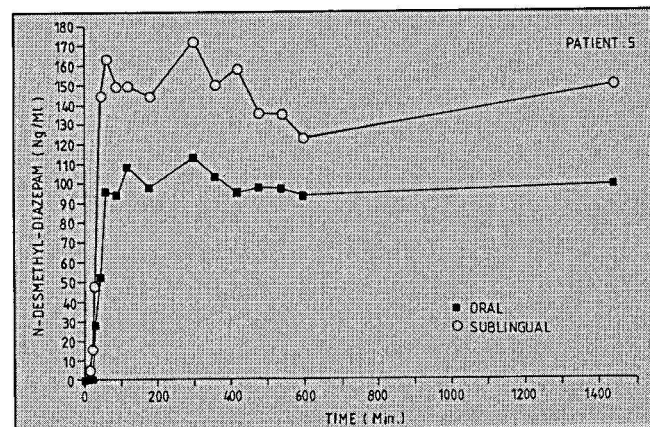
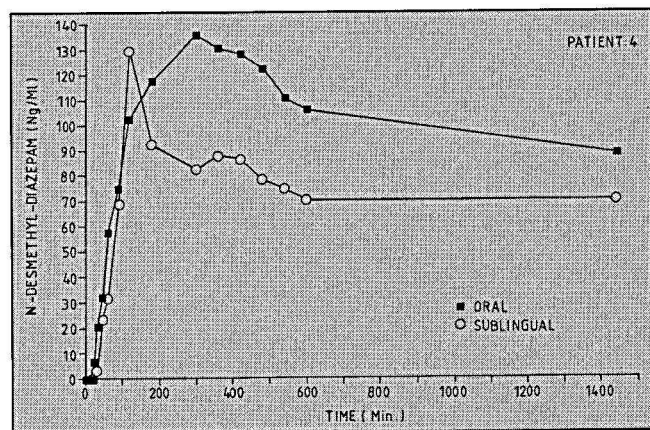
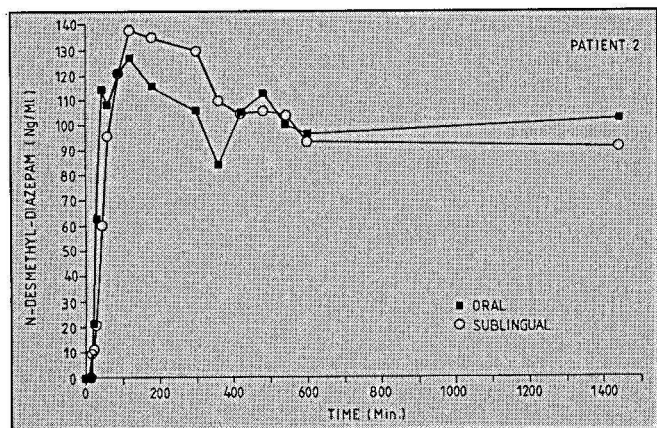
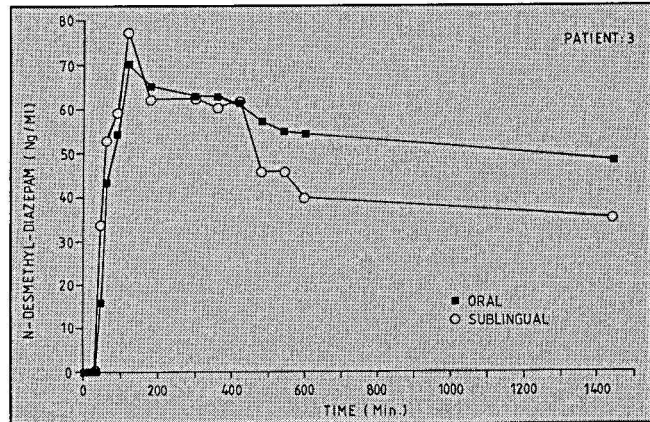
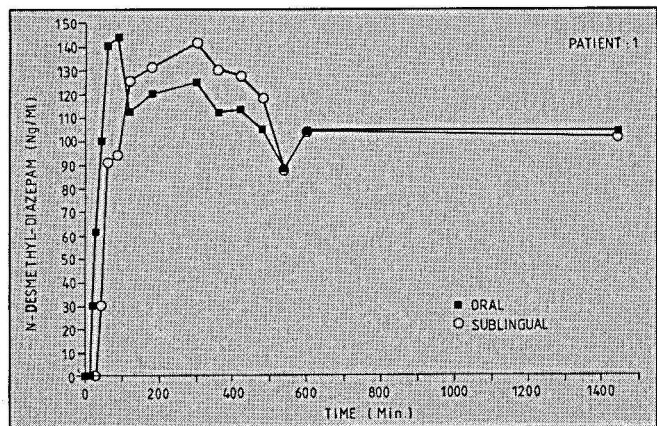


Fig. 1. Time course of N-desmethyl-diazepam serum concentrations after sublingual and oral administrations of prazepam 20 mg to 5 volunteers.

## Discussion

The binding study we performed gave similar results of  $IC_{50}$  for flunitrazepam and diazepam used as control as those obtained by Squires and Braestrup [1977] or Möhler and Okada [1977]. The results obtained for prazepam, N-desmethyl-diazepam and oxazepam suggest that N-desmethyl-diazepam, the major metabolite of prazepam, is much more active than prazepam. Moreover the fact that the concentration of prazepam is much lower than the concentration of N-desmethyl-diazepam at any time after oral administration of prazepam 30 mg [Smith et al., 1979] suggests that prazepam is not active by itself but after metabolization and would be a pro-drug [Ochs et al., 1984].

Using our analytical method no prazepam was detected at a concentration greater than 20 ng/ml (limit of detection) whereas N-desmethyl-diazepam reached concentrations of about 140 ng/ml. Therefore taking into consideration these pharmacodynamic and analytical data, the difference in clinical results of oral and sublingual administrations of prazepam reported by Ansseau et al. [1987] could be correlated with the N-desmethyl-diazepam concentrations.

Unfortunately the comparison of AUC,  $C_{max}$  and  $T_{max}$  for both administrations did not show statistical difference. Nevertheless the last parameter ( $T_{max}$ ) has to be discussed in detail. Indeed, effective levels were reached within 2 h for all subjects and both formulations. But because of the long elimination half-life of N-desmethyl-diazepam ranging from 30 to 120 h [Greenblatt et al., 1981], small variations in concentrations due to physiological factors or analytical method can interfere in the determination of  $T_{max}$ . Nevertheless, taking into consideration this problem, no statistical difference was observed between both formulations. On the other hand, the time needed to detect a significant level (lag-time) of N-desmethyl-diazepam (limit of detection 10 ng/ml) ranged from 22.5 to 45 min but no statistical difference was observed between both formulations. Moreover these data stressed the fact that even after sublingual administration 22.5 min were at least necessary to detect a significant level of N-desmethyl-diazepam whereas the tablet disappeared from the mouth within 5 min after intake. These observations suggest that after sublingual administration a larger amount of prazepam is probably resorbed from the gastrointestinal tract than after oral administration. This suggestion is in good agreement with the clinical observation showing that sublingual prazepam induced a sig-

nificant sedative effect which peaked between 1.5 and 2 h after intake.

The lack of difference between both administrations in their pharmacokinetics can explain in part the results of the 'objective' assessment of vigilance by computerized reaction time that did not show statistical difference. On the other hand this lack of pharmacokinetic difference makes it difficult to interpret the clinical data which pointed out more subjective sedative properties of sublingual administration than oral administration of prazepam 20 mg.

In conclusion, from the pharmacodynamic data we may suggest that prazepam is a pro-drug which is transformed to the active compound N-desmethyl-diazepam. From the pharmacokinetic data, we may suggest that the major part of prazepam administered sublingually is probably absorbed from the gastrointestinal tract. The lack of statistical difference between the two administrations in their pharmacokinetic data hampers a direct pharmacokinetic interpretation of the clinical observation that a single dose of sublingual prazepam appears to exhibit more subjective sedative properties than the same dose of oral prazepam in normal volunteers. Combined pharmacokinetic, pharmacodynamic and physiological data could probably explain such a clinical observation but need to be obtained with a greater number of volunteers.

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## References

- Ansseau, M.; Von Frenckell, R.; Jacqmin, P.: Comparison of sublingual and oral prazepam in normal subjects. I. Clinical data. *Neuropsychobiology* 18: 77-82 (1987).
- De Groot, G.; Grotenhuis-Mullenders, A.M.L.J.: Combined bonded-phase/liquid-liquid extraction as a rapid, specific sample-pretreatment technique for the determination of benzodiazepines in plasma by RP-HPLC. *Top. Forens. Analyt. Toxicol.*, pp. 95-103 (Elsevier, Amsterdam 1984).
- Di Carlo, J.F.; Viau, J.P.; Epps, J.E.; Haynes, L.J.: Biotransformation of prazepam in man. *J. Pharmacol. Ther.* 11: 890-896 (1970).
- Greenblatt, D.J.; Laughren, M.D.; Allen, M.D.; Harmatz, J.S.; Shader, R.I.: Benzodiazepines: a summary of pharmacokinetic properties. *Br. J. clin. Pharmacol.* 1: 35-40 (1981).

- Hirtz, J.: La biodisponibilité: interprétation des résultats expérimentaux et prise de décision. *INSERM* 96: 439-446 (1980).
- Jacqmin, P.; Wibo, M.; Lesne, M.: Classification of benzodiazepine receptor agonists, inverse agonists and antagonists using bicuculline in an in vitro test. *J. Pharmacol.* 17: 139-145 (1986).
- Lowry, O.H.; Rosebrough, N.J.; Farr, A.L.; Randall, R.J.: Protein measurement with the Folin phenol reagent. *J. biol. Chem.* 193: 265 (1951).
- Möhler, H.; Okada, T.: Benzodiazepine receptor: demonstration in central nervous system. *Science* 198: 849-851 (1977).
- Mousah, H.; Jacqmin, P.; Lesne, M.: The quantification of GABA in the cerebrospinal fluid by a radioreceptor assay. *Clinica chim. Acta* 170: 151-160 (1987).
- Mura, P.; Piriou, A.; Fraillon, P.; Papet, Y.; Reiss, D.: Screening procedure for benzodiazepines in biological fluids by high-performance liquid chromatography using a rapid-scanning multi-channel detector. *J. Chromatogr. biomed. Appl.* 416: 303-310 (1987).
- Ochs, R.; Greenblatt, D.; Verlug-Ochs, B.; Locniskar, A.: Comparative single dose kinetics of oxazolam, prazepam and chlorazepate: three precursors of desmethyldiazepam. *J. clin. Pharmacol.* 24: 446-451 (1984).
- Smith, M.T.; Evans, L.E.J.; Eadie, M.J.; Tyrer, J.H.: Pharmacokinetics of prazepam in man. *Eur. J. clin. Pharmacol.* 16: 141-147 (1979).
- Spriet, A.; Beiler, D.: Table of facilitate determination of symmetrical confidence intervals in bioavailability trials with Westlake's method. *Eur. J. Drug Metab. Pharmacokinet.* 2: 129-132 (1978).
- Squires, R.; Braestrup, C.: Benzodiazepines receptors in rat brain. *Nature* 266: 732-734 (1977).
- Vesell, E.S.; Passananti, G.T.; Viau, J.P.: Effects of chronic prazepam administration on drug metabolism in man and rat. *Pharmacology* 7: 197-206 (1972).
- Wagner, J.G.: Fundamentals of clinical pharmacokinetics. Drug Intelligence Publications, Illinois/Illinois (USA), pp. 290-297 (1975).
- Westlake, W.J.: Use of confidence intervals in analysis of comparative bioavailability trial. *J. pharm. Sci.* 61: 1340-1341 (1972).
- Westlake, W.J.: Symmetrical confidence intervals for bioequivalence trials. *Biometrics* 32: 741-744 (1976).

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